

PATENTANSPRÜCHE

1. Method for identifying and/or the producing an effector of a calmodulin-dependent peptidyl-prolyl *cis/trans* isomerase (CaMAP) consisting of the following steps:
 - (a) mixing of appropriate amounts of a CaMAP or a CaMAP peptide fragment/derivative with an appropriate amount of calmodulin or of a calmodulin fragment/derivative in an appropriate reaction solution with and without the effector;
 - (b) adding an appropriate amount of an appropriate CaMAP substrate,
 - (c) measuring CaMAP activity; and
 - (d) detecting that the effector is
 - (i) an inhibitor if the CaMAP activity in the reaction solution with the effector is lower than in the reaction solution without the effector; or
 - (ii) an activator if the CaMAP activity in the reaction solution with the effector is higher than in the reaction solution without the effector.

2. Method for screening and/or producing an effector of a CaMAP consisting of the steps of
 - (a) mixing appropriate amounts of a CaMAP or a CaMAP peptide fragment/derivative with an appropriate amount of calmodulin or a calmodulin fragment/derivative in an appropriate reaction solution with and without a sample containing a single or a multitude of compounds which are candidates for an inhibitor or an activator;
 - (b) adding an appropriate amount of an appropriate CaMAP substrate;
 - (c) measuring CaMAP activity; and
 - (d) detecting that the sample
 - (i) exhibits inhibitory activity if the CaMAP activity in the reaction solution with the sample is lower than in the reaction solution without the sample; or
 - (ii) exhibits activating activity if the CaMAP activity in the reaction solution with the sample is higher than in the solution without the sample.

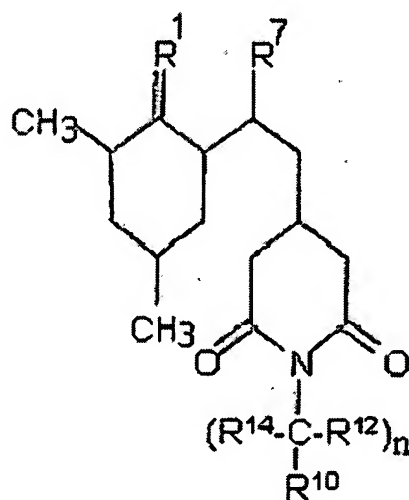
3. Method according to claim 2, further comprising step
 - (e) fractioning of the sample for which inhibitory or activating activity was detected in step (d) and repeating of steps (a) to (d) until the inhibitor or activator contained in the sample is present in purified form.
4. Method according to any one of claims 1 to 3, wherein the CaMAP is selected from the group consisting of the human CaMAPs FKBP36, FKBP37.7, FKBP44, FKBP51, FKBP52 and Cyp40 and enzymes that are listed in the "Swiss-Prot" database corresponding to the denotation used in this database under FKBP66, FKBP42, AIP_HUMAN, AIP_CERAE, AIP_MOUSE, AIPL1_HUMAN, AILP1_RAT, AILP1_MOUSE, AILP1_RABIT, FKB8_HUMAN, FKB8_MOUSE, FKB5_HUMAN, FKB5_MOUSE, FKB4_HUMAN, FKB4_MOUSE, FKB4_RABIT, FKB7_WHEAT, CYP4_BOVIN and CYP4_HUMAN.
5. Method according to any one of claims 1 to 4, wherein the calmodulin or the calmodulin fragment/derivative is selected from the group consisting of

CALM_ACHKL (P15094), CALM_BLAEM (Q9HFX6), CALM_CANAL (P23286), CALM_CAPAN (P93087), CALM_CHLRE (P04352), CALM_DICDI (P02599), CALM_DROME (P07181), CALM_ELEEL (P02594), CALM_EMENI (P19533), CALM_EUGGR (P11118), CALM_FAGSY (Q39752), CALM_HELAN (P93171), CALM_HORVU (P13565), CALM_HUMAN (P02593), CALM_KLULA (O60041), CALM_LYCES (P27161), CALM_LYTPI (P05935), CALM_MAGGR (Q9UWF0), CALM_MAIZE (P41040), CALM_MALDO (P48976), CALM_MEDSA (P17928), CALM_METSE (P02596), CALM_NEUCR (Q02052), CALM_ORYSA (P29612), CALM_PARTE (P07463), CALM_PATSP (P02595), CALM_PHYIN (P27165), CALM_PLAFA (P24044), CALM_PLECO (P11120), CALM_PNECA (P41041), CALM_PYUSP (P11121), CALM_SCHPO (P05933), CALM_SOLTU (P13868), CALM_SPIOL (P04353), CALM_STJA (P21251), CALM_STRPU (P05934), CALM_STYLE (P27166), CALM_TETPY (P02598), CALM_TETTH (Q05055), CALM_TRYBB (P04465), CALM_TRYCR (P18061), CALM_WHEAT (P04464), CALM_YEAST (P06787), Q9UWF0, Q02052, P19533, AAL89686,

Q7M510, Q96TN0, P27165, AAG01043, P02593, Q7T3T2, Q40302, O02367, Q95NR9, Q9UB37, AAH54805, AAH54973, AAL02363, AAH59427, AAH59500, AAH54600, AAH53150, AAH50926, AAH45298, AAH44434, AAP88918, AAP35501, AAP35464, BAC56543, AAC83174, AAD55398, AAC63306, AAD45181, AAH21347, BAC40168, BAB28631, BAB28319, BAB28116, BAB23462, AAH58485, AAH51444, AAH47523, P07181, Q7QGY7, Q8STF0, AAO25039, AAM50750, AAK61380, BAB89360, O94739, P02594, Q9D6G4, O16305, Q96HK3, P11120, O96102, P21251, Q9U6D3, Q8X187, O93410, AAR10240, P11121, Q9XZP2, Q42478, AAQ01510, P17928, P93171, O97341, O96081, AAD10244, AAM81203, AAA34238, AAA34014, AAA34013, P02596, P93087, Q43699, CAD20351, BAB61916, BAB61915, AAF65511, P02595, P59220, P27162, Q93VL8, Q39447, Q94801, AAQ63462, AAQ63461, AAM81202, BAB61918, BAB61917, BAB61914, BAB61913, BAB61912, BAB61911, BAB61910, BAB61909, AAG27432, AAG11418.

6. Method according to any one of claims 1 to 5, wherein the appropriate reaction solution contains bivalent ions selected from the group consisting of Zn^{2+} , Cu^{2+} , Co^{2+} , Ni^{2+} , Mn^{2+} , Ca^{2+} and/or Mg^{2+} at a concentration of 0,1 to 20 mM.
7. Method according to any one of claims 1 to 6, wherein the appropriate reaction solution has a pH of between pH 5 and pH 10.
8. Method for identifying and/or producing an effector of a CaMAP consisting of the steps
 - (a) mixing appropriate amounts of a constitutively active CaMAP in an appropriate reaction solution with and without effector;
 - (b) adding an appropriate amount of an appropriate CaMAP substrate;
 - (c) measuring CaMAP activity; and
 - (d) detecting that the effector is
 - (i) an inhibitor if the CaMAP activity in the reaction solution with the effector is lower than in the reaction solution without the effector; or

- (ii) an activator if the CaMAP activity in the reaction solution with the effector is higher than in the reaction solution without the effector.
9. Method according to any one of claims 1 to 8, wherein steps (a) and (b) are interchanged.
 10. Method according to any one of claims 1 to 9, wherein the detection is carried out by spectroscopic or radioactive methods.
 11. Method according to any one of claims 1 to 10, wherein the method is a high-throughput method.
 12. Method according to any one of claims 1 to 11, further comprising step
 - (f) formulating the identified and/or produced effector with a pharmaceutically acceptable carrier or solvent.
 13. Compound identified according to any one of methods 1 to 11, wherein the effector is a cycloheximide derivative having the general formula (1):



in which n is an integer from 1 to 20; R^{12} independently is a hydrogen atom, an alkyl residue or an aryl residue,

R¹ is selected from an oxygen atom, a sulfur atom, or the groups NR², NOR² and N-NR²R³, wherein

(a) R² and R³, independently from each other, are a hydrogen atom, aryl or alkyl, respectively, which can optionally be interrupted by O, S, NH, NR⁵, aryl, heteroaryl, cycloalkyl, heterocycloalkyl or can optionally be substituted by R⁶, or

(b) R² and R³, together, are C₁-C₆-alkylene, which can optionally be interrupted by O, S, NH, NR⁵, aryl, heteroaryl, cycloalkyl or heterocycloalkyl or can optionally be substituted by R⁶, wherein

R⁵ is an alkyl residue or an aryl residue,

R⁶ stands for a hydrogen atom, alkyl, aryl, OR⁵, C(O)OR⁵, CN, F or Cl, wherein R⁵ is defined as above,

R⁷ is a -OH, -OR⁹, -OC(O)R⁹, -OC(S)R⁹, -OC(O)NHR⁹ or -OC(S)NHR⁹ residue, wherein

R⁹ is an alkyl residue which can optionally be interrupted by O, S, NH, NR⁵, aryl, heteroaryl, cycloalkyl or heterocycloalkyl or can optionally be substituted by R⁶ as defined above, or alternatively

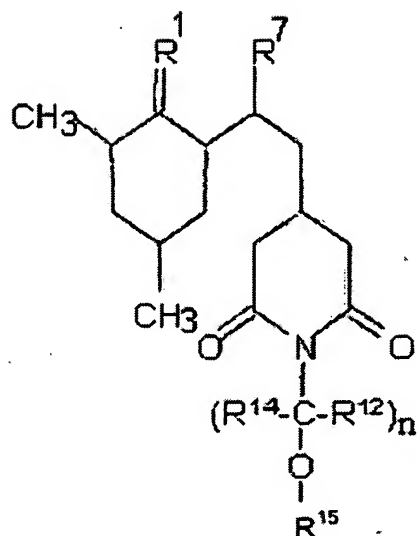
R⁹ is an aryl residue which can optionally be interrupted by O, S, NH or NR⁵ or can optionally be substituted by R⁶ as defined above,

R¹⁰ is a -NHR², -NR²R³, -C(O)OR², -C(S)OR², -C(O)NR²R³, -CN, -NR²C(O)NR²R³, -OC(O)NR²R³, -NR²C(S)NR²R³, -OC(S)NR²R³, or OR², C(O)NHR¹¹ residue, wherein R² and R³ are defined as above,

R¹¹ stands for an amino acid residue or an oligopeptide residue and

R¹⁴ is an alkyl residue or an aryl residue.

14. Compound identified according to any one of methods 1 to 11, wherein the effector is a cyclohexamide derivative having the general formula (1) in which n is an integer of 1 to 20 and exhibiting an ether group between R¹⁵ and the complete molecule as illustrated in formula (2) below,



R^1 is selected from an oxygen atom, a sulfur atom, or the groups NR^2 , NOR^2 and $N-NR^2R^3$, wherein

- (a) R^2 and R^3 , independently from each other, are a hydrogen atom, aryl or alkyl, respectively, which can optionally be interrupted by O, S, NH, NR^5 , aryl, heteroaryl, cycloalkyl, heterocycloalkyl or can optionally be substituted by R^6 , or
- (b) R^2 and R^3 , together, are C_1 - C_6 -alkylene, which is optionally interrupted by O, S, NH, NR^5 , aryl, heteroaryl, cycloalkyl or heterocycloalkyl or can optionally be substituted by R^6 , wherein
 R^5 is an alkyl residue or an aryl residue,
 R^6 stands for a hydrogen atom, alkyl, aryl, OR^5 , $C(O)OR^5$, CN, F or Cl,
 wherein R^5 is defined as above,

R^7 is a $-OH$, $-OR^9$, $-OC(O)R^9$, $-OC(S)R^9$, $-OC(O)NHR^9$ or $-OC(S)NHR^9$ residue, wherein

R^9 is an alkyl residue which can optionally be interrupted by O, S, NH, NR^5 , aryl, heteroaryl, cycloalkyl or heterocycloalkyl or can optionally be substituted by R^6 as defined above, or alternatively

R^9 is an aryl residue which can optionally be interrupted by O, S, NH or NR^5 or can optionally be substituted by R^6 as defined above,

R^{11} stands for an amino acid residue or an oligopeptide residue,

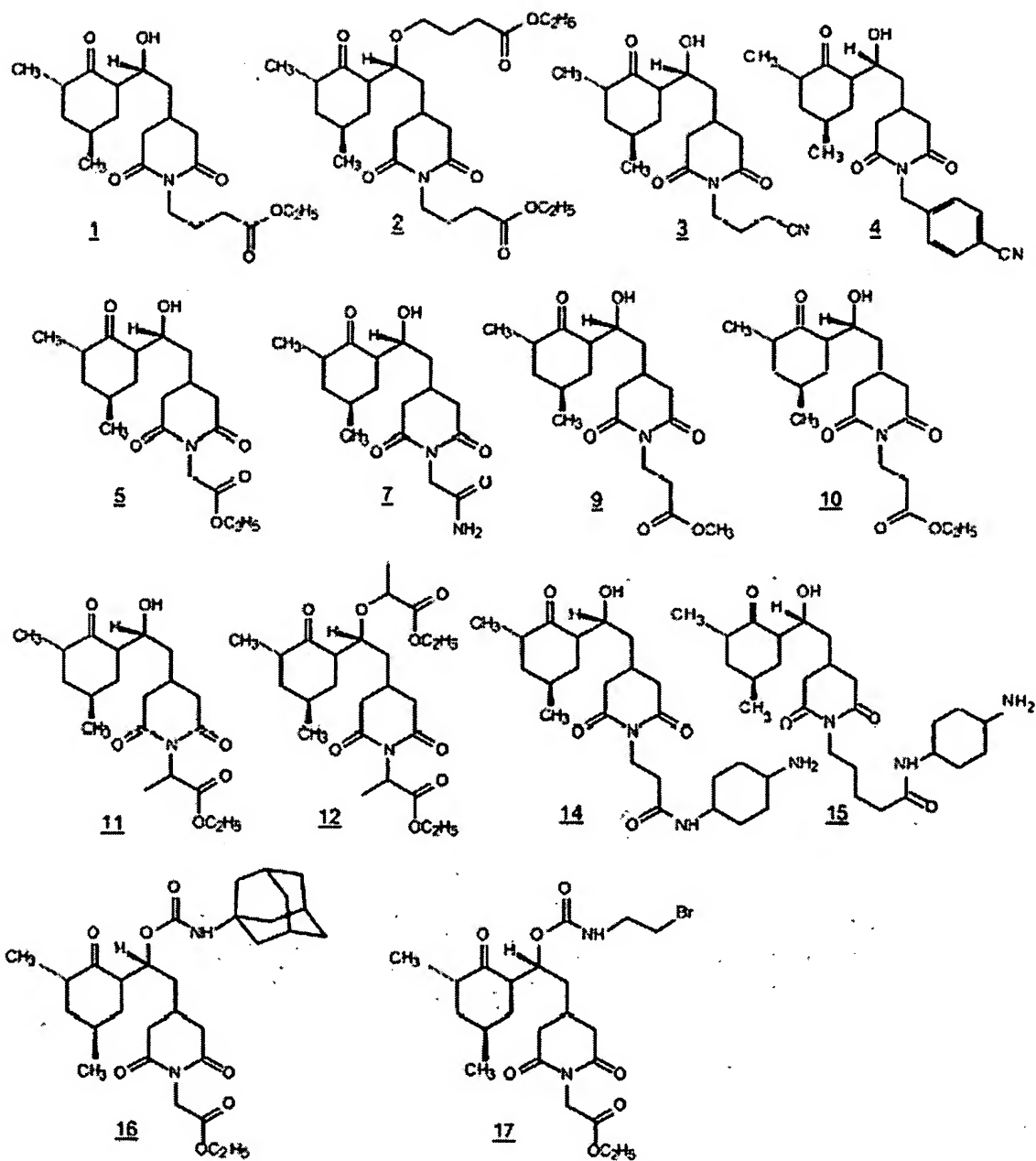
R^{14} is a hydrogen atom, and

R^{15} is a hydrogen atom, an alkyl or an aryl residue.

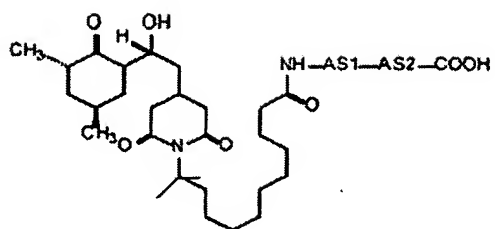
15. Compound according to claim 13 or 14 having the above-identified formula (1) for which the following applies:

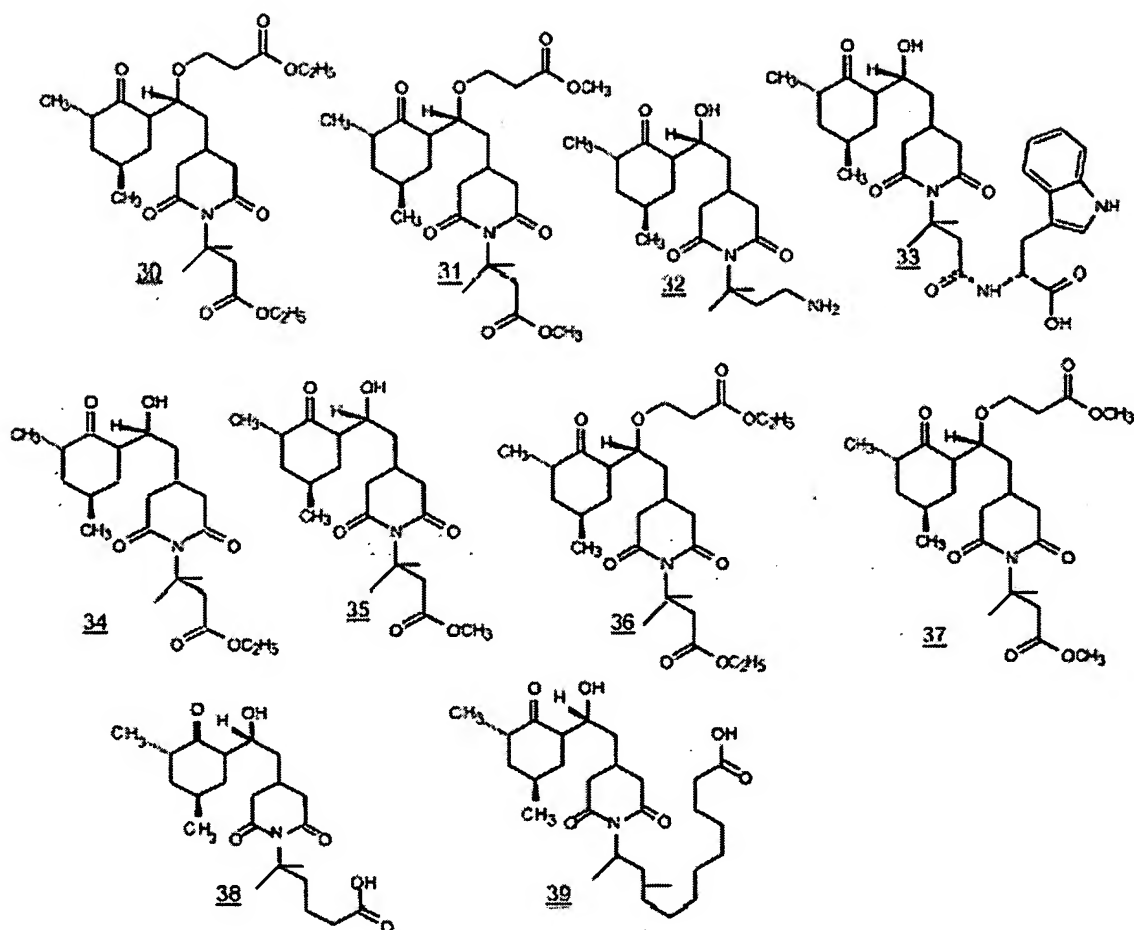
- (a) $n = 1, 2, 3$; $R^1 = O$; $R^7 = OH, O(CHR^{12})_nR^{10}, OC(O)CH_3$; $R^{10} = C(O)OCH_3, C(O)OC_2H_5, CN, C(O)NH_2$,
- (b) $n = 3-10$; $R^1 = O$; $R^7 = OH$; $R^{10} = C(O)NHR^{11}$, $R^{11} =$ amino acid residue, oligopeptide residue,
- (c) $n = 1, 2, 3$; $R^1 = O$; $R^7 = OH, O(CHR^{12})_nR^{10}$; $R^{10} = C(O)OCH_3, C(O)OC_2H_5, CN, C(O)NH_2$,
- (d) $n = 1, 2, 3$; $R^1 = NOH, N-NHPh, N-NHCH_3, N\text{-alkyl}, N\text{-benzyl}$; $R^7 = OH, O(CHR^{12})_nR^{10}$; $R^{10} = C(O)OCH_3, C(O)OC_2H_5, CN, C(O)NH_2$,
- (e) $n = 1, 2, 3$; $R^1 = O$; $R^7 = OH, O(CHR^{12})_nR^{10}, OC(O)NH\text{-alkyl}, OC(O)NH\text{-cycloalkyl}, OC(O)NH\text{-aryl}$; $R^{10} = C(O)OCH_3, C(O)OC_2H_5, CN, C(O)NH_2$.

16. Compound according to claims 13 to 15 with the above-identified formula:



compound	amino acid residue AS1	amino acid residue AS2
<u>18</u>	alanine	alanine
<u>19</u>	valine	alanine
<u>20</u>	tryptophan	alanine
<u>21</u>	isoleucine	alanine
<u>22</u>	methionine	alanine
<u>23</u>	glycine	alanine
<u>24</u>	alanine	valine
<u>25</u>	valine	valine
<u>26</u>	tryptophan	valine
<u>27</u>	isoleucine	valine
<u>28</u>	methionine	valine
<u>29</u>	glycine	valine





17. Effector according to any one of claims 1 to 16, optionally with a pharmaceutically acceptable carrier or solvent.
18. Use of an effector according to any one of claims 1 to 16 for the preparation of a medicament for the treatment of tumour diseases.
19. Use of an effector according to any one of claims 1 to 16 for the preparation of a medicament for the inhibition or attenuation of transplant rejection or for the treatment of neurodegenerative diseases.
20. Kit, comprising CaMAP or a peptide fragment/derivative as described in claim 1 or 4 and calmodulin or a calmodulin fragment/derivative as described in claim 1 or 5, one or more buffer solutions and /or one or more substrates.